Robin Productivity in the Housatonic River Watershed

Berkshire County, Massachusetts

PREPARED FOR

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Table of Contents

Table of Contents

Tables

Figures

1. Introduction

On behalf of the General Electric Company (GE), ARCADIS studied robin productivity in the Housatonic River watershed during the 2001 breeding season. The purpose of this report is to describe that study's methods, results and conclusions. The objectives of this study were to: a) document reproductive output of robins; b) evaluate exposure of eggs and young to polychlorinated biphenyls (PCBs); and c) evaluate relationships between exposure and reproductive output.

The general approach to this study involved: a) identifying as many robin nests as possible both within the Housatonic River floodplain and in reference areas beyond foraging distance of the floodplain; b) monitoring clutch size, hatching success and fledging success in each of those nests; c) collecting one egg and one 7-day old nestling from each nest, to the extent feasible, and analyzing those samples to determine concentrations of PCBs; and d) evaluating data for statistical relationships between PCB exposure and measures of reproductive success, and for differences in reproductive performance in the exposed (target) and reference populations. A similar approach was employed in a songbird study that was conducted for GE in 1993 (Henning, Ebert et al. 1997), except that the 1993 study evaluated all bird species for which nests were found and did not include any chemical analyses.

2. Methods

This section details the methods employed in the robin productivity study, including the definition of study areas, nest searching techniques, nest measurements, nest monitoring, egg and nestling sample collections, sample processing, chemical analyses, chain-of-custody procedures, database development and statistical analyses.

2.1 Definition of Study Areas

The study area for the robin productivity study encompassed the Housatonic River watershed in Berkshire County, Massachusetts (MA). Permission was obtained to access six areas of public land, as well as land privately owned by GE, the Town of Lenox and the Eastover Resort. The study focused on land with suitable breeding habitat for robins (i.e., predominantly early to mid-successional forests with proximity to edges). Suitable and accessible land within the watershed was defined as either the target area or one of several reference areas.

The target area was restricted to the 10-year floodplain of the Housatonic River, from the confluence of the East Branch and West Branch of the Housatonic River in Pittsfield, MA to Woods Pond Dam in Lenox, MA. This reach of the river measures approximately 10 river miles (16 km).

All reference areas were public lands within the Housatonic River watershed: Peru Wildlife Management Area, Peru State Forest, Middlefield State Forest, October Mountain State Forest and Hinsdale Flats Wildlife Management Area. All reference areas were located well beyond the foraging range (984 ft or 300 m per Knupp, Owen et al. 1977) of target robins inhabiting the 10-year floodplain of the Housatonic River. As discussed below, absence of exposure of the reference population to PCBs was confirmed through chemical analyses of egg and nestling samples.

2.2 Nest Searching

Each area was thoroughly searched for nests by foot. Robin nests were identified based on the appearance of the nests, as well as the presence and behavior of robins nearby. Robin nests are primarily built out of grass or other vegetation and possess an inner mud layer lined with fine grass (Baicich and Harrison 1997; Sallabanks and James 1999). Upon locating a nest, it was checked to see whether it contained eggs or nestlings. Nests located too high to be viewed directly were checked either using a rear-view bicycle mirror attached to an extendable pole to view the contents of the nest (preferred method), or by reaching into the nest to feel for and count eggs or nestlings.

If a nest had a fresh, wet mud lining or contained eggs or nestlings, it was considered active, and measurements were recorded as discussed in Section 2.3. If no adults, eggs or nestlings were present when the nest was found, the nest was observed from a distance for up to 20 minutes to see if an adult appeared. If no adults appeared, the location of the nest was recorded and it was visited again within three to five days to check for adult activity or eggs and to confirm that it was an active robin nest.

The behavior of adult robins in the field was assessed as an indicator of the likelihood of a nest being located nearby. This behavioral evaluation considered whether: males were singing, males were defending the territory against other males, a pair was observed feeding together, robins were behaving in a covert manner, robins were calling defensively, females were carrying nest materials and/or robins were carrying food. If it appeared likely that there was a robin nest nearby, the robins were observed until: the nest was visually located, the robins remained out of sight and could not be relocated or such time passed that it did not seem likely that they would lead the

observer to the nest. If the nest was found prior to the addition of the mud layer, the nest location was noted and visited three to five days later to determine if it had been completed.

2.3 Nest Measurements

Each nest was assigned a three-digit number in sequential order, regardless of whether it was located within the target or a reference area. A numbered wooden stake was driven into the ground, a compass bearing was taken, and the distance from the stake to the nest was visually estimated. The height of the nest above the ground was also visually estimated.

Information regarding the nest location relative to potential predator accessibility was recorded based on three factors. Conspicuousness of the nest was considered by rating the volume of deciduous and coniferous foliage within one meter of the nest as low, medium or high. Accessibility for ground predators was evaluated by rating the proximity of the nest to large branches as low (distal to main trunk, on small branches), medium (on middle sized branches) or high (proximal to main trunk). The isolation of the nest tree or shrub was assessed by ranking the nest tree's isolation as low (part of continuous layer of foliage), medium (isolated within continuous vegetation or at the edge of continuous vegetation) or high (completely isolated from adjacent vegetation). It should be noted that this index was only relevant to sight-based predators and was based on the premise that such predators use the same habitat features that human observers use to locate nests. This index was not meant to be a measure of vulnerability to predators that hunt by scent, such as some mammalian predators.

Nest locations were determined using a hand-held global positioning system unit (GPS, Garmin GP-12), for use in relocating nests during subsequent field activities. Locations of nests were hand-drawn on maps. The location of each nest was subsequently confirmed using a Trimble TSC 1 Asset Surveyor GPS unit, which is more accurate than the hand-held unit.

2.4 Nest Monitoring

Once a nest was determined to be active, it was monitored every three days, with a few exceptions. In order to minimize the likelihood of flushing females from nests when it might put eggs at risk of temperature stress, nests were not visited when air temperatures were below 50°F (10°C) or when it was raining heavier than a light

drizzle. A few nests were not revisited for up to six days because of weather or scheduling conflicts.

Generally, on the first visit after the nest was determined to be active, parental attentiveness was assessed. The following parameters were recorded: the distance at which the female flushed off the nest as the researcher approached, the number of calls during a one-minute period with a researcher at the nest, the number of approaches by the adult male or female to a mirror pole or stick held over the nest during a one-minute period and the closest distance of an adult robin to the nest during the one-minute period.

On all visits to each nest, the numbers of eggs and nestlings were recorded. Viewing was enhanced through the use of a rear-view bicycle mirror attached to an extendable pole, a ladder and/or tree climbing. The development of nestlings was recorded during each visit, based on characteristics such as whether eyes were open and the extent of feather development. Incidental observations were also recorded, such as whether the female flushed from the nest and the presence of adults calling near the nest or carrying food near the nest. Nests located too high to be observed directly or with a mirror on a pole were observed from the ground with binoculars for up to 15 minutes for any adult activity. Such observation periods were terminated once evidence of parental nest attendance or nestling presence was observed. Such nests determined to be active were subsequently inspected using a ladder where feasible; otherwise, such nests were excluded from data analysis.

Nest outcome was also recorded, in the event that nest success was precluded by: a) never being initiated; b) being abandoned; or c) being depredated. These three outcomes were determined as follows. Nests that were never initiated were defined as such because either: a) mud or grass linings were never added; b) adults were never observed in the vicinity of the nest; and/or c) eggs were never observed in the nest. Nests were defined as abandoned if they had been confirmed as active during the 2001 breeding season, but activity ceased prior to completion of the reproductive cycle and there was no evidence of disturbance of the nest. For example, reference nest 037 had been active and monitored over a period of 13 days, but on the fifth visit was found with four dead three-day-old nestlings and no evidence of damage to the nest. Another example of a nest that was defined as abandoned was target nest 020, in which eggs had been incubated for approximately one week, when on the third visit the eggs were found to be present but cold and there was no evidence of disturbance of the nest. Consistent with Davidson and Knight (2001), nests were defined as depredated if all eggs or nestlings disappeared before the young were old enough to fledge. If young

were absent but old enough to fledge, nests were classified as complete (i.e., successful).

2.5 Egg Collection

State and federal scientific collection permits were secured prior to collecting any specimens. One viable egg was collected from nests containing four or more eggs, wherever feasible. Eggs that had been incubated for at least 10 days were targeted for collection, based on an estimate from previous visits of when the clutches were complete and incubation had begun. A random egg was collected from each nest by arbitrarily assigning an egg a value of one and then sequentially numbering the remaining eggs clockwise. A random number was selected from a preprinted list of random numbers to select the egg for collection. Viability of the selected egg was determined by candling. If the selected egg was not viable, it was replaced and an alternate randomly selected egg was collected and candled to determine viability. In addition, in some cases nonviable eggs were also collected for analysis or possible future analysis.

Disposable latex gloves were worn during all egg collection activities. Eggs were placed in a ventilated plastic container lined with unused, clean bubble-wrap for padding and the collection time was recorded. Eggs were transported to the GE workspace for processing within two hours of collection.

2.6 Nestling Collection

Nestlings were collected from nests with three or more nestlings. Nestlings were collected at approximately 7 days of age. Age was estimated from hatching date or, if hatching date was uncertain, from feather development and the timing of when eyes opened. Nestlings were not collected from all nests where three or more eggs hatched because some nests were depredated before nestlings were 7 days old. At the time when nestlings were estimated to be 7 days old, the largest nestling was collected in an effort to ensure consistent treatment across all nests.

Disposable latex gloves were worn during all nestling collection activities. Nestlings were removed from the nest by hand and placed in a ventilated plastic container lined with unused, clean aluminum foil. The time of collection was recorded. Nestlings were transported to the GE workspace for processing within two hours of collection.

2.7 Sample Processing

Egg and nestling samples were processed in GE workspace in Pittsfield, MA. The entire workspace was cleaned in advance of initiating the study, and the absence of PCBs was confirmed through sampling and analysis. Separate workspace, storage space, freezers and tools were used for target and reference samples to prevent cross-contamination. All processing equipment was cleaned and rinsed with acetone between samples. Prior to processing any samples on a given day, the accuracy of the balance (200 G Scout II Balance, O'Haus Corporation) was verified using 5 g, 10 g, and 20 g check weights. The balance consistently gave readings within 0.1% of the check weights' expected weights.

Samples were processed as follows. The processing time was recorded. Eggs and nestlings were first weighed in the field collection container and weights were recorded to the nearest hundredth of a gram. The sample was then removed from the container and the empty container was weighed, to allow the sample weight to be determined based on the difference between the two weights.

After weighing, eggs were opened using a scalpel with a clean, unused blade. The contents were transferred to chemically precleaned glass sample containers. External anatomy was evaluated for deformities. Eggshells and scalpel blades were discarded and the egg sample containers were labeled and immediately placed in a freezer. Following weighing, nestlings were placed in chemically precleaned sample containers and killed via decapitation. External anatomy of nestlings was also examined for deformities. The nestling sample containers were labeled and placed in a freezer.

Samples were labeled with a unique sample identification number, collection time and date, initials of the researchers who collected the sample, processing date and time and initials of the researcher who processed the sample. Target area and reference area samples were stored in separate freezers at approximately -20°C. Samples were maintained frozen until transferred to the analytical laboratory courier.

Distilled water blanks were prepared by filling chemically precleaned sample containers approximately half-full with distilled water, frozen in the same freezers as field-collected samples and shipped to the analytical laboratory along with field-collected samples. Samples were transported to the analytical laboratory in coolers containing blue ice. A laboratory courier drove samples from Pittsfield, MA to the analytical laboratory in Schenectady, NY.

2.8 Chemical Analyses

Egg and nestling samples were analyzed by Northeast Analytical Environmental Lab Services of Schenectady, New York (NEA). The feathers, beaks and legs of nestling samples were removed prior to initiating chemical analysis. All samples were analyzed for PCBs using SW-846 Method 8082, which targets individual Aroclors and quantifies total PCBs. All samples were also analyzed for percent lipids using Method NE158_1.SOP. Because only the nestling samples contained sufficient sample mass, only the nestling samples were also analyzed for percent moisture using ASTM Method D2974.

2.9 Chain-of-Custody Procedures

Chain-of-custody (COC) forms were initiated in the field at the time of sample collection, and accompanied samples from the field to the GE workspace for processing and then to NEA for chemical analysis. A single COC form was used for all samples shipped on a given day. COC forms specified sample identification codes, matrix, date and time sampled, analyses requested for each sample and the dates and times that custody of the samples was exchanged between people. The field staff and the laboratory's courier signed the COC form upon relinquishing the samples to the laboratory. Both ARCADIS and NEA retained copies of the COC forms. COC tape was affixed to the lids of the individual samples as an additional measure to verify that appropriate custody of samples was maintained.

2.10 Database Development

Pertinent data for each nest were entered into a single database in Excel 2000 (Microsoft Corporation, Redmond, WA) to allow simultaneous evaluation of parameters pertaining to predation, breeding cycle, productivity and chemistry. Intermediate calculations were also performed on a number of measures, as described below.

- The number of nests completed was calculated as the total number of nests that fledged at least one young.

- The percent of nests completed was calculated as the number of nests that fledged at least one young divided by the total number of active nests monitored.

- Analytical results below the detection limit were assigned a proxy concentration equal to one-half the detection limit.

- Concentrations of PCBs in eggs and nestlings were normalized to lipid content by dividing the PCB concentration by the percent lipids.

- For the three measures of predator accessibility – conspicuousness, accessibility and isolation – values of 1, 2, and 3 were assigned to the qualitative descriptors of low, medium, and high[1]. A single metric was calculated as the arithmetic mean of the three measures. The two observers' indices were also averaged prior to entering a final index into the database. High predator accessibility indices reflect greater accessibility of the nest to predators.

- The four measures of nest defense – vocalizations, approaches, flushing distance and minimum distance from pole – were evaluated both as independent measurements and as a single metric, based on the number of vocalizations, the number of approaches, the inverse of the flushing distance, and the inverse of the minimum distance from the pole. These four measures were averaged to yield a single metric. High nest defense indices reflect stronger defensive behavior by the robins.

- Clutch size was calculated as the total number of eggs laid in successful nests, as well as in nests that were depredated or abandoned after the start of incubation.

- The number of nonviable eggs was counted in successful nests based on the difference between the clutch size and the number of young hatched.[2] The eggs that were collected were included in the count of the number of nonviable

[1] Because foliage volume is inversely related to conspicuousness, low foliage volume was assigned a score of 3, while high foliage volume was assigned a score of 1.

[2] However, if there was evidence of depredation of the unhatched egg, it was recorded as depredated, rather than nonviable.

eggs, based upon direct observation of whether the egg was viable upon dissection.

- The proportion of nonviable eggs was calculated as the total number of nonviable eggs divided by the total number of eggs laid in successful nests. Again, the viability of collected eggs was based on direct observation through dissection.

- The egg date was converted from a calendar date to a serial number, in which the date January 1, 1900 was assigned the value 1. This conversion was necessary to complete subsequent statistical testing of egg dates. However, egg dates are presented in this report as the calendar date, for ease in interpretation. Nests that were discovered after egg laying had begun were recorded as having unknown egg dates and were excluded from the evaluation of egg date or incubation period. While it would have been possible to estimate the egg dates based on expected incubation periods (as reported in the literature), to do so would have biased calculation and analysis of incubation period as a measure of effect.

- Incubation period was calculated as the duration of time between when the clutch was complete (i.e., all eggs had been laid) and when eggs hatched. In several cases, nests were not visited on the exact day that incubation began or eggs began hatching. In such cases, these days were interpolated as the midpoint between the days when the nests were monitored. The incubation period was not calculated for nests that were discovered after the clutch was complete. Again, although incubation period could have been estimated based on reports from the literature, to do so would have compromised the objectivity of incubation period as a measure of effect.

- The number of nestlings hatched per successful nest was calculated in two ways.

    - First, the "range-low" number hatched was counted, ignoring the likelihood that the viable eggs that were collected would have hatched, had they not been collected. This method underestimates the number of nestlings hatched per successful nest, because at least some of the viable eggs that were collected may have hatched had they not been collected. The bias is greater among target nests than reference nests, because more eggs and nestlings were collected from target nests.

- Second, the "range-high" number of nestlings that would have hatched had the eggs not been collected was counted. In this case, it was assumed that the collected eggs that were nonviable would not have hatched and that the collected eggs that were viable would have hatched. This method may overestimate the number of nestlings hatched per successful nest, because it does not account for embryo mortality that might have occurred among the collected eggs, had they not been collected.

Because the former method may be biased low and the latter biased high, the two methods define the ranges of nestlings hatched per successful nest.

- Hatching success was calculated as the number of young hatched divided by the number of eggs present just before hatching, for all nests with data (Mayfield 1975). Because this method does not account for the collected viable eggs that would have hatched had they not been collected, it underestimates what the hatching success would have been in the absence of egg collections. Again, this bias is greater for target nests than for reference nests, due to the larger number of target eggs collected.

- Nestling period was calculated as the duration of time between hatching and fledging for all successful nests. Again, it was sometimes necessary to estimate the hatch date or the fledge date as the midpoint between the two days when the nests were visited immediately before or after either event.

- The number of nestlings fledged per successful nest was calculated in two ways.

  - First, the "range-low" number fledged was counted, ignoring the possibility that some of the viable eggs and nestlings that were collected would have fledged had they not been collected. This method likely underestimates the number of nestlings fledged per successful nest, because at least some of the viable eggs and nestlings that were collected probably would have fledged had they not been collected. The bias is greater among target nests than reference nests due to the larger number of target eggs and nestlings collected.

  - Second, the "range-high" number of nestlings that would have fledged had the eggs and nestlings not been collected was counted. In this

case, it was assumed that the collected eggs that were nonviable would not have hatched and that both the collected eggs that were viable and the nestlings would have hatched. This method may overestimate the number of nestlings fledged per successful nest, because it does not account for embryo and nestling mortality that might have occurred among the collected eggs and nestlings, had they not been collected.

Because the former method may be biased low and the latter biased high, the two methods define the ranges of nestlings fledged per successful nest.

- Fledging success was calculated as the ratio of young fledged to young hatched for all successful nests. As in the calculation of hatching success, the outcome of collected eggs and nestlings was ignored, likely biasing this measure low.

- Nest success was calculated by the Mayfield (1975) method, using CONTRAST software.

An independent reviewer verified the accuracy of 100% of the data entry and intermediate calculations.

2.11 Statistical Analysis

Upon completing the development of the database, statistical analyses were conducted using Power and Sample Size for Windows (PASS) (NCSS, Kaysville, Utah). Student's t-tests and analyses of variance (ANOVAs) were performed to determine if statistically significant differences were observed between reference and target samples for factors related to exposure and effects. Mann-Whitney U-tests were performed for measures based on proportional data. Both one-tailed and two-tailed tests were conducted for all statistical comparisons. Statistical comparisons were considered to be significant at an alpha level (α) of 0.05. Power was calculated based on Student's t-tests. However, due to the limitations of this approach for the present application (as discussed below), bioequivalence tests of means were also conducted for the most ecologically relevant endpoints, using the methodology described by Hintze (2000) and Blackwelder(1982).

Spearman correlation coefficient matrices were also calculated to determine the strength of relationships between wet weight PCB concentrations and measures of

reproductive success and between lipid-normalized PCB concentrations and measures of reproductive success.

The accuracy of all statistical analyses was independently verified using an alternate statistical software package (Winks, ver. 4.62, TexaSoft, Cedar Hill, TX).

3. Results

A total of 106 active robin nests were located and monitored during the 2001 breeding season. Of these, 44 were located in the reference area and 62 were located in the target area. The numbers of nests depredated, abandoned and completed are shown in Table 1. Of the target nests monitored, 29% fledged at least one young (i.e., were successful), while 25% of the reference nests were successful. The vast majority of nests that were not successful were depredated.

Nine viable eggs and eleven nestlings were collected from target nests and analyzed for PCBs. Two viable eggs and six nestlings were collected from reference nests and analyzed for PCBs.

Findings for measures of exposure and measures of effects are presented below. One-tailed t-test results are tabulated for measures of exposure, while two-tailed t-test results are tabulated for measures of effects.

3.1 Measures of Exposure

Table 2 presents the analytical results for total PCBs detected in viable eggs and nestlings collected from the target and reference areas, as well as basic summary statistics (minimum, maximum, mean, median, geometric mean, sample size and standard error)[3]. Wet weight concentrations of PCBs in target area viable eggs ranged from 5.04 mg/kg to 170 mg/kg, while those in reference area eggs ranged from 0.07 to 0.24 mg/kg. Wet weight concentrations of PCBs in target area nestlings ranged from 0.09 mg/kg to 43.7 mg/kg, while those in reference area nestlings ranged from 0.03 to 0.06 mg/kg.

The statistical analysis of the nestling and egg chemistry results, presented in Table 3, confirms that the robins defined as the target population were significantly more

[3] For consistency, comparisons of analytical results for eggs include only viable eggs. The PCB results for nonviable eggs are presented in a footnote within Table 2.

exposed to PCBs than were the robins defined as the reference population. The wet weight concentration of PCBs in target eggs (mean=83.6 mg/kg) was much greater than in reference eggs (mean=0.153 mg/kg), a difference that is statistically significant (p=0.00283). The lipid-normalized concentration of PCBs in target eggs (mean=2,217 mg/kg) was also much greater than in reference eggs (mean=2.41 mg/kg), a difference that is also statistically significant (p=0.00453). Similarly, for nestlings, the wet weight concentration of PCBs in target nestlings (mean= 11.9 mg/kg) was greater than in reference nestlings (mean= 0.0372 mg/kg), a difference that is statistically significant (p=0.0153). The lipid-normalized concentration of PCBs in target nestlings (mean=523 mg/kg) was greater than in reference nestlings (mean=1.82 mg/kg), a difference that is also statistically significant (p=0.0124). PCBs were not detected in any of the blank samples.

3.2 Measures of Effects

This study evaluated measures of reproductive effects related to parental attentiveness, fertility, survival to hatching, development and survival to fledging. The results from the statistical analyses of these measures are presented in Tables 4 through 8. The null hypothesis tested through these analyses was that the means from the target area population and the reference area population were equal. The results of one-tailed tests yielded the same conclusions as those presented below based on two-tailed tests, although power was lower for the one-tailed tests than for the two-tailed tests.

Parental attentiveness was indirectly evaluated based on rates of abandonment and depredation of nests, numbers of nestlings that were abandoned or depredated, defensive behavior, and accessibility of the nests to predators. The results of statistical analyses of parental attentiveness are presented in Table 4. Although target robins appear to have performed better than reference robins for all measures of parental attentiveness, only two of these differences are statistically significant. A lower percentage of nests were abandoned in the target area (mean=1.5%) compared to the reference area (mean=6.5%), a difference that was not statistically significant (p=0.153). The incidences of nest depredation in the target area (mean=63%) and the reference area (mean=65%) were not significantly different (p=0.831). A lower number of target young were either abandoned or depredated (mean=0.636) compared to reference young (mean=1.84), a difference that was statistically significant (p=0.0132) and opposite of that which would be predicted by an exposure-related effect.

Additional analyses were conducted to explore the potential for confounding in the comparison of depredation rates as a result of differences in timing of observations relative to the nesting cycles of target and reference populations. Theoretically, susceptibility to depredation may vary within the nesting cycle, such that depredation rates could differ in two populations if they were observed at different points in the nest cycles. For this reason, the Mayfield index offers a more reliable measure of nest success, compared to depredation rate. Possible seasonal effects were considered by calculating the Mayfield index for the target area nests with the 25 earliest egg dates (defined as "early nests") and the 25 latest egg dates (defined as "late nests"). There were no statistically significant differences between early target nests (daily predation rate (dpr)=5.66%, standard error (SE)=1.53%, sample size (n)=229.5 exposure days) and late target nests (dpr=4.15%, SE=0.91, n=481.5 exposure days) (chi-square=0.6274, degrees of freedom (df)=1, p=0.4283). However, there was a statistically significant difference between the dpr of late target nests (dpr=4.15%, SE=0.91, n=481.5 exposure days) and reference nests (dpr=7.68%, n=338.5 exposure days) (chi-square=4.252, df=1, p=0.0392), suggesting that differences in depredation were related to differences in habitat and predator density in the target and reference areas, to a greater extent than to seasonal differences in observation periods.

The predator accessibility indices for target nests (mean=2.07) and reference nests (mean=2.13) were not significantly different (p=0.436). The component parts of the predator accessibility index – conspicuousness, accessibility, and isolation – were also considered independently. The conspicuousness of target nests (mean= 3.97) was also lower than that of reference nests (mean=4.06), a difference that was not statistically significant (p=0.0655). The accessibility of target nests (mean=4.54) was lower than that of reference nests (mean=5.10), a difference that was statistically significant (p=0.024) but opposite that which would be predicted by an exposure-related effect on behavior. The isolation of target nests (mean=3.90) was greater than that of reference nests (mean=3.59), a difference that was not statistically significant (p=0.239). Spearman correlation analyses indicate no significant relationships between whether a nest was depredated and either the composite accessibility index (correlation coefficient = - 0.106, p=0.279) or the component parts of the index [(conspicuousness: correlation coefficient = 0.0547, p=0.577), (accessibility: correlation coefficient = - 0.171, p=0.0795), and (isolation: correlation coefficient = 0.0351, p=0.721)]. Hence, neither the index nor its component parts proved to be useful predictors of likelihood of predation. Additionally, given that the reference nests were more accessible to predators and were more likely to be depredated than the target nests, there is no evidence that observed differences in nest accessibility or depredation were related to exposure to PCBs. It is more likely that the differences are attributable to differences in

habitat (e.g., density of vegetation) and predator densities in the target and reference areas.

The nest defense indices for target nests (mean=93.4) and reference nests (mean=90.5) did not differ significantly (p=0.901). The component parts of the nest defense index – number of vocalizations, number of approaches, flushing distance and minimum distance from pole – were also considered independently. The number of vocalization of target robins (mean=26.0) was lower than that of reference robins (mean=29.9), a difference that was not statistically significant (p=0.591). The number of approaches of target robins (mean=1.74) was also lower than that of reference robins (mean=2.33), a difference that was not statistically significant (p=0.316). The flushing distance of target robins (mean=11.8) was greater than that of reference nests (mean=8.73), a difference that was not statistically significant (p=0.270). The minimum distance from pole for target robins (mean=27.7) was greater than that of reference nests (mean=26.5), a difference that was not statistically significant (p=0.867). Spearman correlation analyses indicate no significant relationships between whether a nest was depredated and either the composite nest defense index (correlation coefficient = -0.0418, p=0.806) or the component parts of the index [(vocalizations: correlation coefficient = - 0.0892p=0.434), (approaches: correlation coefficient = 0.0226, p=0.843), (flushing distance: correlation coefficient = 0.00, p=1.00), or (minimum distance from pole: correlation coefficient = - 0.00917, p=0.949]. Hence, neither the index nor its component parts is a useful predictor of likelihood of predation. Given that the reference nests were more likely to be depredated than the target nests, there is no evidence that observed differences in nest defense or depredation were related to exposure to PCBs. Again, it is more likely that the differences are attributable to differences in habitat (e.g., density of vegetation) and predator densities in the target and reference areas.

Fertility was evaluated based on clutch size and number of nonviable eggs, as presented in Table 5. The clutch sizes for target nests (mean=3.56) and reference nests (mean=3.31) were not significantly different (p=0.141). The numbers of nonviable eggs per successful target nest (mean=0.471) and per successful reference nest (mean=0.222) also were not significantly different (p=0.399). The proportion of nonviable eggs in successful target nests (mean=0.109) and in successful reference nests (mean=0.556) was not significantly different (p=0.284).

Survival to hatching was evaluated based on the incubation period, range-low and range-high numbers of nestlings hatched per successful nest and hatching success, as detailed in Table 6. The incubation periods for target area nests (mean=14.2 days) and

reference area nests (mean=14.0 days) were not significantly different (p=0.0775). The range-low numbers of nestlings hatched per successful target area nest (mean=2.89) and per successful reference area nest (mean=2.64) were not significantly different (p=0.337). The range-high number of nestlings hatched per successful target nest (mean=3.22) was greater than the number hatched per successful reference nest (mean=2.73), a difference that was statistically significant (p=0.0362) and opposite that which would be predicted by an exposure-related effect. Hatching success in target area nests (mean=93%) and in reference area nests (mean=96%) also did not differ significantly (p=0.555).

The development of embryos and nestlings was evaluated by examining external anatomy and weighing all egg and nestling samples. No morphological abnormalities were observed in any of the specimens examined. As shown in Table 7, weights of target eggs (mean=5.49 g) and reference eggs (mean=5.93 g) were not significantly different (p=0.518). The weights of target nestlings (mean=48.2 g) and reference nestlings (mean=46.9 g) also were not significantly different (p=0.815).

As presented in Table 8, survival to fledging was evaluated based on nestling period, range-low and range-high numbers of nestlings fledged per successful nest, fledging success and Mayfield nest success. The nestling periods for target nests (mean=13.9 days) and reference nests (mean=13.6 days) were not significantly different (p=0.221). The range-low numbers of nestlings fledged per successful target nest (mean=2.22) and per successful reference nest (mean=1.91) were not significantly different (p=0.145). The range-high numbers of nestlings fledged per successful target nest (mean=3.17) was greater than the number fledged per successful reference nest (mean=2.45), a difference that was statistically significant (p=0.00759) and opposite that which would be predicted by an exposure-related effect.

Although fledging success was higher for target robins (mean=98%) than for reference robins (mean=91%), these differences were not statistically significant (p=0.288). The overall Mayfield dpr for the reference area was 7.68% (SE=1.44%, n=338.5 exposure days), which corresponds to an overall nest success or survival rate of 10.7% (assuming a 28-day egg-laying plus incubation plus nestling period). This estimate does not include several nests that were abandoned. The overall dpr for the target area was 4.64% (SE=0.8%, n=711 exposure days), which corresponds to an overall survival rate of 26.4%. These differences were not statistically significant (chi-square=3.4057, df=1, p=0.0650).

Spearman correlation coefficients were calculated to explore whether any statistically significant relationships exist between PCB exposure and reproductive outcome. These results are presented in Tables 9 and 10 for wet weight and lipid-normalized PCB concentrations, respectively. Because the predator accessibility index, the nest-defense index and their component parts did not prove to be meaningful predictors of depredation, they were omitted from this analysis. As illustrated in Table 9, there were no statistically significant correlations ($p < 0.05$) between wet weight concentrations of PCBs in eggs or nestlings and any of the measures of effects. Spearman correlation coefficients were also calculated on a lipid-normalized basis; again no statistically significant correlations between exposure and productivity were observed (Table 10).

4. Discussion

During the 2001 breeding season, 62 active robin nests were found and monitored in the target area and 44 active robin nests were found and monitored in the reference area. These sample sizes are consistent with those of other studies in which robin nests were monitored (e.g., Champagne 1975; Davidson and Knight 2001; Fluetsch and Sparling 1994; Howell 1942; Johnson, Mack et al. 1976; Kemper and Taylor 1981; Ortega, Ortega et al. 1997, Morneau, Lepine et al. 1995, Yen, Klaas et al. 1996).

As detailed above, despite substantial differences in the degree to which target and reference area robins were exposed to PCBs, this study provides no evidence of adverse effects on any stage of reproduction in robins as a result of exposure to PCBs. The majority of statistical tests were not significant ($p<0.05$) and the few that were significant were opposite of that which would be predicted by an exposure-related effect. The mean concentrations of PCBs in target eggs and nestlings were more than two orders of magnitude higher than in reference eggs and nestlings. Nonetheless, both populations of robins performed very well and in many ways, target area robins exhibited superior reproductive performance (i.e., $p>0.5$ or results significant and opposite of that which would be predicted by an exposure-related effect). Specifically, in the target area, fewer nests and young were abandoned or depredated, clutch sizes were higher, the proportion of nests that fledged at least one young was higher, the number of nestlings fledged per successful nest was higher, fledging success was higher, and overall nest success was higher. In some cases, including range-high numbers of nestlings hatched and fledged, the performance of target nests was statistically significantly better than that of reference nests (i.e., opposite that which would be predicted by an exposure-related effect). As further discussed below, there is no evidence that PCBs have adversely affected the productivity of robins inhabiting the 10-year floodplain.

Data on measures of exposure (i.e., PCB concentrations in eggs and nestlings) clearly demonstrated that target robins were hundreds of times more highly exposed than target robins. Where sufficient data were available to allow testing for statistical significance, the differences in concentrations of PCBs were statistically significant ($p<0.05$). These differences in PCB concentrations are also biologically significant because they demonstrate that the target and reference populations of robins were distinct. The concentrations of PCBs observed in reference area eggs and nestlings were generally consistent with those reported for robin eggs and nestlings collected from four agricultural areas in Canada (which ranged from 0.031 mg/kg to 0.227 mg/kg for eggs and ranged from 0.0063 mg/kg to 0.159 mg/kg for nestlings) (Harris, Wilson et al. 2000). For these reasons, the very low and/or nondetectable concentrations of PCBs in the reference robins may be defined as the background level of exposure of robins to PCBs. The absence of detectable concentrations of PCBs in blank samples suggests that cross-contamination of samples did not occur during sample collection, processing or analysis.

Some of the measures of effects evaluated are more ecologically relevant than others. When considering whether PCBs are likely to have population-level effects, the most pertinent question that can be evaluated through nest monitoring studies relates to survival to fledging. Although clutch size certainly influences production rates, this measure has less ecological relevance than the number of young fledged because it does not account for the many challenges posed to embryos between the time of egg laying and fledging. That is, two nests with very different initial clutch sizes may yield the same number of young and vice versa; of these measures, it is the number of young that survives to fledging that most directly affects the sustainability of the population. We measured a number of endpoints that influence productivity, in order to better understand the full potential for PCBs to be linked to adverse reproductive effects. However, the following discussion focuses on those endpoints that are most relevant to the sustainability of local populations: proportion of nests that fledged at least one young, number of young fledged per nest, fledging success and nest success.

Of the 62 target nests monitored, 29% fledged at least one young. Of the 44 reference nests monitored, 25% fledged at least one young. Both values are well within the range (8.3% to 75%) reported for natural and reference populations by various researchers (Brehmer and Anderson 1992; Fluetsch and Sparling 1994; Howard 1974; Howell 1942; Johnson, Mack et al. 1976; Kemper and Taylor 1981; McLean, Smith et al. 1986; Morneau, Lepine et al. 1995; Ortega, Ortega et al. 1997; Young 1955).

The range-low number of target nestlings fledged per successful nest (mean=2.22) and the range-low number of reference nestlings fledged per successful nest (mean=1.91) were not significantly different (p=0.145) (Figure 1). However, the range-high number of nestlings fledged is significantly greater in target nests (mean=3.17) than in reference nests (mean=2.45) (p=0.00759), a finding that is opposite that which would be predicted by an exposure-related effect (Figure 2). Other robin studies have reported between 1.0 and 4.2 nestlings fledged per successful nest (Beaver 1980; Champagne 1975; Decarie, DesGranges et al. 1993; Fluetsch and Sparling 1994; Gill, Wilson et al. 2000; Johnson, Mack et al. 1976; Kemper and Taylor 1981; Knupp, Owen et al. 1977; Mason 1943; Morneau, Lepine et al. 1995; Ortega, Ortega et al. 1997; Tweist 1965; Young 1955). Hence, survival to fledging among Housatonic River floodplain robins was within natural ranges reported in the literature regardless of whether or not the collected eggs and nestlings were included in the count.

Fledging success appears to have been higher for target nests (mean=98%) than for reference nests (mean=91%), but this difference was not statistically significant (p=0.202). Figure 3 helps illustrate the differences in fledging success between target and reference nests, showing substantial differences between mean values but overlapping standard errors. Fledging success reported in other robin studies varied between 62% and 100% (Brehmer and Anderson 1992; Gill, Wilson et al. 2000; Kemper and Taylor 1981; Ortega, Ortega et al. 1997; Rondeau and Desgranges 1995; Young 1955), suggesting that fledging success for both target and reference nests was within the range of natural variability.

The Mayfield estimate of nest success was higher for target nests (26.4%) than for reference nests (10.7%), a difference that was not statistically significant (chi-square=3.4057, df=1, p=0.0650) and opposite that which would be predicted by an exposure-related effect (Figure 4). Hence, there is no evidence that overall nest success was adversely affected by exposure to PCBs. Target area nest success calculated using the Mayfield method was near the lower end of the range reported by other researchers (18%-90%), while reference area nest success was lower than values reported in the literature (Fluetsch and Sparling 1994; Knupp, Owen et al. 1977; Morneau, Lepine et al. 1995; Niles 1985; Sallabanks and James 1999; Yahner 1983; Yen, Klass et al. 1996; Young 1955). Because the Mayfield estimate of nest success primarily describes depredation, this broad range of published values is likely attributable to geographic and habitat variability in predator densities and availability of alternative prey, as well as timing of observations. Yen, Klaas et al. (1996) reported that survival rate among early season robin nests (37.11%) was significantly lower ($p<0.05$) than that of late season nests (66.74%). As previously discussed, results for the Housatonic study rule

out seasonal effects within the timing of this study as a primary influence on differences in predation rates. Because the nest success results for the target and reference populations within the Housatonic River watershed were more similar to one another than they were to data from the literature, it appears that habitat differences, variability in predator densities and availability of alternative prey may have greater influence on nest success than either the degree of exposure to PCBs or seasonal effects. Differences between nest success in the Housatonic River watershed and other locations are not attributable to exposure to PCBs, because the unexposed reference population exhibited the lower nest success. For these reasons, the observed differences between the target and reference populations in nest success do not appear to be biologically significant.

The duration of the nestling period is also indirectly relevant to fledging success, since prolonged nestling periods could increase risks from predation. However, the nestling periods for target nests (mean=13.9 days) and reference nests (mean=13.6 days) were not significantly different (p=0.221), even though they were slightly higher than the range of natural variability reported in the literature (12 to 13.4 days) (Sallabanks and James 1999; Yen, Klaas et al. 1996; Young 1955). Furthermore, compared to the reference nests, target nests had lower depredation despite slightly longer nestling periods. Hence, there were no biologically significant differences in nestling periods for reference and target nests.

All remaining measures of effects are secondary indicators of productivity, in that they are less ecologically relevant than those discussed above. For the secondary measures of effects that relate to parental attentiveness, fertility, survival to hatching and development, target nests performed as well as or better than reference nests. Where data are available in the literature, data for target and reference nests were generally within the range of reported natural variability. For example, clutch sizes for both target (mean=3.56) and reference (mean=3.31) populations were consistent with nationwide clutch sizes (2.8 to 3.6) (Beaver 1980; Fluetsch and Sparling 1994; Gill, Wilson et al. 2000; Howard 1967; Howard 1974; Howell 1942; Johnson 1969; Johnson, Mack et al. 1976; Kemper and Taylor 1981; Kendeigh 1942; Klimstra and Stieglitz 1957; Knupp, Owen et al. 1977; Martin 1973; Mehner 1958; Morneau, Lepine et al. 1995; Rondeau and Desgranges 1995; Tweist 1965; Yahner 1983; Yen, Klaas et al. 1996; Young 1955). Likewise, the incubation periods for target (mean=14.2 days) and reference (mean=14.0 days) nests were virtually the same and appear to be within the range of natural variability as reported in the literature (12 to 14 days) (Kaufman 1996; Kendeigh 1952; Howell 1942; Manning 1982; Sallabanks and James 1999; Yen, Klaas et al. 1996; Young 1955).

The findings for hatching success were also qualitatively consistent with those of the 1993 study of productivity of songbirds within the Housatonic River watershed (Henning, Ebert et al. 1997). However, it is not appropriate to directly compare the results from the 1993 and 2001 studies because the Mayfield method was not employed in 1993 and because the sample sizes were considerably lower in 1993 than in 2001.

The weights of target and reference eggs (means = 5.49 g and 5.93 g, respectively) were not statistically significantly different and were within the range of natural variability (4.1 g to 7.13 g) reported in the literature (Carey, Garber et al. 1983; Howell 1942; Knupp, Owen et al. 1977; Manning 1982; Sallabanks and James 1999). The large variation reported in the literature may be attributable to timing of weighing, since Manning (1982) demonstrated that robin eggs rapidly lose weight during incubation. Manning (1982) also showed that humidity and precipitation influence the rate of weight loss, while Carey, Garber et al. (1983) demonstrated a direct relationship between egg mass and barometric pressure. Due to the highly variable nature of egg masses, this measure probably is not useful for judging potential effects of environmental pollutants.

As indicated in Tables 4 through 8, the power of rejection of the null hypothesis for the above measures of effects based on the conventional hypothesis testing was relatively low for most measures of effects. This finding is expected, in light of the relatively high p-values. For these analyses, power is not the most relevant statistic. Power is defined as the probability of rejecting the null hypothesis when the null hypothesis is in fact false (i.e., a correct rejection). The low power in the case of this study was an artifact of the limitations of conventional statistics for biological testing, in which one is effectively trying to "prove" the null hypothesis. Another way of looking at this situation is to consider the similarity of the target and reference means for measures of effects. When two means are very close, as in the case of the measures of effects, it is far more difficult to prove a difference between them. When two means are very different, as in the case of the measures of exposure, it is relatively easy to discern that difference. In part for these reasons, the use of retrospective power analyses in wildlife studies has been criticized by some researchers (Steidl, Hayes et al. 1997).

Despite the relatively low power for most measures of effects evaluated using conventional hypothesis testing, several other factors contribute to high certainty in the conclusion that there were no adverse impacts on robin productivity from exposure to PCBs. These factors, discussed below, include concurrence among outcomes for all measures of effects, evidence from the literature that differences are well within the

range of natural variability, bioequivalence testing results, comparisons of 95% upper confidence limits (UCLs), and lack of any statistically significant correlations between degree of exposure to PCBs and productivity outcomes.

First, there was strong agreement among all of the measures of effects that there are no adverse effects. None of the measures of effects provided any evidence of impaired reproduction and there was absolutely no relationship between the degree of exposure to PCBs and the reproductive outcome. The concurrence among the multiple endpoints evaluated enhanced the defensibility of the overall conclusions. The importance of consistency among the outcomes of multiple endpoints in judging causality is discussed elsewhere (USEPA 1992; Suter 1993; Suter, Efroymson et al. 2000; Hill 1965; Menzie, Henning et al. 1996).

Second, confidence in the lack of PCB-related effects on robin productivity was further bolstered by the observation that the clutch sizes, numbers of young fledged per nest, and nest success for target and reference nests were similar to each other and within the range of data reported in the literature. Hence, the reproductive outcome results in this study for both the target area population and the reference area population were within the range of natural variability for robin populations in areas unaffected by PCBs.

Third, bioequivalence testing yielded statistical results that were both more powerful and more meaningful than conventional hypothesis testing. Specifically, when conventional hypothesis testing is applied to biological data, the objective is to "prove" the null hypothesis; this contradicts the very definition of the null hypothesis (i.e., it can only be rejected). As noted by Blackwelder (1982), the p-value "is a measure of evidence against the null hypothesis, not for it, and insufficient evidence to reject the null hypothesis does not imply sufficient evidence to accept it." Bioequivalence testing resolves this difficulty by testing the null hypothesis of whether there is a specified difference (delta), defined in terms of a potentially biologically relevant difference, between the target and the reference populations. Hence, for biological studies, the bioequivalence results are more meaningful than those of the conventional hypothesis testing.

In this case, the null hypotheses tested through the bioequivalence approach were that the target area mean numbers of young hatched or fledged per nest were more than one-half of a nestling less than the reference area mean, and that the mean hatching success and fledging success for the target area were more than 20% lower than that of the reference area. These deltas were selected because 0.5 is within the range of natural variability (as discussed above) and because rejecting a 20% difference is as powerful a

result as is generally expected for ecological field studies (see Suter, Cornaby et al. 1995). The results of the bioequivalence tests are presented in Table 11. In all cases tested, the null hypothesis (i.e., that there is a biologically relevant difference between target and reference populations) was rejected with $p<0.05$ and relatively high power (0.62 to 0.98). Given the power of the analyses when the null hypothesis was appropriately framed, there was a very low probability of "Type II errors" (i.e., false negatives) in this study.

Fourth, the lack of effect was further supported by comparisons of UCLs on the means. Table 12 compares the reference area and target area means and 95% UCLs for all measures of exposure and effects. The comparisons of 95% UCLs were entirely consistent with the comparisons of means: in all cases where the target mean was greater than the reference mean, the target 95% UCL was also greater than the reference 95% UCL. Likewise, in all cases where the target mean was less than the reference mean, the target 95% UCL was also less than the reference 95% UCL. This observation suggests consistency in outcomes both at the central tendency and at the high end of the data distributions.

Finally, correlation coefficients provided no evidence of a relationship between the degree of exposure to PCBs and any of the measures of effects. The Spearman correlation coefficient was selected as a statistical test because, compared to the Pearson correlation coefficient, it is less influenced by outliers, unequal variances, non-normality, and non-linearities (Hintze 2000). There were no statistically significant or strong correlations between any measures of PCB exposure and any of the primary or secondary measures of reproductive success.

It is also worth noting that there were some differences in habitat between the target and reference robins, in that the reference nests were at higher elevation and thawed later than the target area. Consequently, the two sets of nests developed under different conditions; in general, reference nests lagged by two weeks at all stages of the reproductive cycle. However, if this temporal difference influenced productivity, it would be expected that reference robins experienced more favorable conditions for egg and nestling survival (warmer temperatures, greater cover of nests, more abundant food supply), which would be expected to improve reference area reproductive success relative to reproduction of target area robins. Therefore, this potential confounding factor does not change the conclusion of this study.

Additionally, one potential experimental bias associated with repeated visits to a nest is that birds can become habituated to the presence of observers and modify their

behavior accordingly. For example, some birds might become less aggressive or vocal toward observers as the frequency of nest visits increases. However, the potential effects of observer habituation were probably the same at the target and reference areas due to similar nest visit frequencies. Furthermore, Ortega, Ortega et al. (1997) demonstrated that even much more intrusive monitoring programs did not, in and of themselves, adversely affect reproduction in robins. For these reasons, we do not believe that the monitoring influenced the success of the nests or had a differential effect between target and reference nests.

5. Summary

We examined robin productivity within the Housatonic River watershed by evaluating the exposure of eggs and young to PCBs, monitoring the reproductive cycle of robins, and evaluating the relationship between the reproductive outcome and exposure. One hundred and six active robin nests were located in the target and reference areas and were monitored approximately every three days throughout the breeding season. Egg and nestling samples were collected from active nests for PCB and lipid analyses. Concentrations of PCBs in target and reference specimens differed significantly, providing strong evidence that the populations defined as target and reference truly were exposed to differing levels of PCBs. The only statistically significant differences in measures of effects were opposite that which would be predicted by an exposure-related effect. Observed variability in productivity was well within the range of natural background. The outcomes of all endpoints consistently failed to provide evidence of adverse effects from PCBs; uncertainty in the overall conclusions is minimized with such concurrence in results. Bioequivalence tests confirmed the absence of statistically significant and biologically significant differences between target and reference robins. Correlation coefficients showed no evidence of a relationship between PCB exposure and any measure of reproductive effects.

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Table 1
Summary of Nests Observed

	Total Number of Nests	Number of Nests Depredated	Number of Nests Abandoned	Number of Nests Completed [a]	Percent of Nests Complete [a]
Reference	44	30	3	11	25%
Target	62	43	1	18	29%

[a] completed nests fledged at least one young

Table 2
Concentration of Polychlorinated Biphenyls (PCBs) in Viable Egg and Nestling Samples

		Viable Eggs [a]			Nestlings		
Nest	Area	PCB Concentration (mg/kg)	Lipids (%)	Lipid-Normalized PCB Concentration (mg/kg)	PCB Concentration (mg/kg)	Lipids (%)	Lipid-Normalized PCB Concentration (mg/kg)
009	Target	NS	NS	NS	10.2	4.12	248
012	Target	NS	NS	NS	3.90	3.16	123
013	Target	NS	NS	NS	8.10	1.18	686
022	Target	6.7	4.66	144	2.91	3.23	90.1
023	Target	18.4	4.71	391	7.74	1.96	395
031	Reference	NS	NS	NS	0.0277 U	2.21	1.26 U
032	Target	NS	NS	NS	0.0919	2.45	3.75
033	Target	5.04	2.96	170	NS	NS	NS
035	Target	NS	NS	NS	0.704	3.03	23.2
036	Target	NS	NS	NS	4.05	1.20	338
037	Reference	NS	NS	NS	0.0574	1.64	3.50
043	Target	162	5.07	3195	43.7	2.52	1734
045	Target	NS	NS	NS	41.7	2.26	1845
049	Target	150	4.89	3067	NS	NS	NS
056	Target	103	2.04	5049	NS	NS	NS
061	Reference	0.238	7.54	3.16	NS	NS	NS
067	Reference	NS	NS	NS	0.0271 U	2.18	1.25 U
069	Target	51.4	4.76	1080	NS	NS	NS
077	Reference	NS	NS	NS	0.0273 U	1.60	1.71 U
088	Target	NS	NS	NS	7.29	2.70	270
092	Reference	NS	NS	NS	0.056	3.39	1.65
099	Reference	0.0675 U	4.07	1.66 U	NS	NS	NS
108	Target	86.3	5.10	1692	NS	NS	NS
110	Target	170	3.90	4359	NS	NS	NS
111	Reference	NS	NS	NS	0.0275 U	1.79	1.54 U
Target	Minimum	5.04	2.04	144	0.0919	1.18	3.75
	Maximum	170	5.10	5049	43.7	4.12	1845
	Mean	83.6	4.23	2127	11.9	2.53	523
	Median	86.3	4.71	1692	7.29	2.52	270
	Geometric Mean	47.2	4.08	1159	4.71	2.37	199
	Sample Size (n)	9	9	9	11	11	11
	Standard Error	22.3	0.4	619.2	4.7	0.3	197.7
Reference	Minimum	0.0675	4.07	1.66	0.0271	1.60	1.25
	Maximum	0.238	7.54	3.16	0.0574	3.39	3.50
	Mean	0.153	5.81	2.41	0.0372	2.14	1.82
	Median	0.153	5.81	2.41	0.0276	1.99	1.59
	Geometric Mean	0.127	5.54	2.29	0.0349	2.06	1.69
	Sample Size (n)	2	2	2	6	6	6
	Standard Error	0.085	1.735	0.748	0.006	0.273	0.346

NS - not sampled

U - non-detect; value given represents one-half the detection limit

[a] In addition, two nonviable eggs were collected from the target area and analyzed for lipids and PCBs. The nonviable egg collected from nest 009 contained 37.5 mg/kg PCBs and 4.22% lipids. The nonviable egg collected from nest 012 contained 7.38 mg/kg PCBs and 5.91% lipids.

Table 3
Measures of Exposure

	PCBs in Eggs	PCBs in Nestlings	Lipid-Normalized PCBs in Eggs	Lipid-Normalized PCBs in Nestlings
Number of Reference Samples (n)	2	6	2	6
Number of Target Samples (n)	9	11	9	11
Reference Mean (mg/kg)	0.153	0.0372	2.41	1.82
Target Mean (mg/kg)	83.6	11.9	2127	523
Reference Standard Error	0.0856	0.00616	0.750	0.347
Target Standard Error	22.3	4.70	619	198
Null Hypothesis (H_o)	$R_t \leq R_r$	$R_t \leq R_r$	$R_t \leq R_r$	$R_t \leq R_r$
p-Value	0.00283	0.0153	0.00447	0.0124
Power (alpha=0.05)	0.960	0.757	0.930	0.791
Decision	Reject H_o	Reject H_o	Reject H_o	Reject H_o

Table 4
Measures of Effects: Parental Attentiveness

	Proportion of Nests Abandoned	Proportion of Nests Depredated	Number of Young Abandoned or Depredated	Predator Accessibility			
				Predator Accessibility Index	Conspicuousness	Accessibility	Isolation
Number of Reference Samples (n)	46	46	19	49	49	49	49
Number of Target Samples (n)	68	68	22	68	68	68	68
Reference Mean	6.5%	65%	1.84	2.13	4.06	5.10	3.59
Target Mean	1.5%	63%	0.636	2.07	3.97	4.54	3.90
Reference Standard Error	0.0369	0.0711	0.422	0.0469	0.580	0.169	0.177
Target Standard Error	0.0147	0.0589	0.136	0.0491	0.481	0.169	0.178
Null Hypothesis (H_o)	$R_t = R_r$	$R_t = R_r$	$R_t = R_r$	$R_t = R_r$	$R_t = R_r$	$R_t = R_r$	$R_t = R_r$
p-Value	0.154	0.832	0.0132	0.436	0.712	0.024	0.239
Power (alpha=0.05)	0.297	0.0552	0.720	0.121	0.0655	0.620	0.216
Decision	Do not reject H_o	Do not reject H_o	Reject H_o	Do not reject H_o	Do not reject H_o	Reject H_o	Do not reject H_o

	Nest Defense				
	Nest Defense Index	Vocalizations	Approaches	Flushing Distance	Minimum Distance to Pole
Number of Reference Samples (n)	13	33	33	15	21
Number of Target Samples (n)	21	46	46	26	31
Reference Mean	90.5	29.9	2.33	8.73	26.5
Target Mean	93.4	26.0	1.74	11.8	27.7
Reference Standard Error	18.2	6.02	0.480	1.56	4.67
Target Standard Error	13.9	4.51	0.360	2.20	6.03
Null Hypothesis (H_o)	$R_t = R_r$	$R_t = R_r$	$R_t = R_r$	$R_t = R_r$	$R_t = R_r$
p-Value	0.901	0.591	0.316	0.270	0.867
Power (alpha=0.05)	0.0517	0.0832	0.169	0.193	0.053
Decision	Do not reject H_o	Do not reject H_o	Do not reject H_o	Do not reject H_o	Do not reject H_o

Table 5
Measures of Effects: Fertility

	Clutch size	Number of Nonviable Eggs per Successful Nest	Proportion of Nonviable Eggs
Number of Reference Samples (n)	29	9	9
Number of Target Samples (n)	39	17	17
Reference Mean	3.31	0.222	0.0556
Target Mean	3.56	0.471	0.109
Reference Standard Error	0.100	0.222	0.0557
Target Standard Error	0.126	0.174	0.0400
Null Hypothesis (H_o)	$R_t = R_r$	$R_t = R_r$	$R_t = R_r$
p-Value	0.141	0.399	0.284
Power (alpha=0.05)	0.312	0.131	0.116
Decision	Do not reject H_o	Do not reject H_o	Do not reject H_o

Table 6
Measures of Effects: Survival to Hatching

	Incubation Period (days)	Range-Low Number of Nestlings Hatched per Successful Nest	Range-High Number of Nestlings Hatched per Successful Nest [a]	Hatching Success
Number of Reference Samples (n)	3	11	11	18
Number of Target Samples (n)	10	18	18	22
Reference Mean	14.0	2.64	2.73	96%
Target Mean	14.2	2.89	3.22	93%
Reference Standard Error	0	0.244	0.195	0.0304
Target Standard Error	0.200	0.137	0.129	0.0354
Null Hypothesis (H_o)	$R_t = R_r$	$R_t = R_r$	$R_t = R_r$	$R_t = R_r$
p-Value	0.0775	0.337	0.0362	0.555
Power (alpha=0.05)	0.0183	0.156	0.566	0.084
Decision	Do not reject H_o	Do not reject H_o	Reject H_o	Do not reject H_o

[a] number of nestlings predicted to have hatched if viable eggs had not been collected

Table 7
Measures of Effects: Development

	Egg Weight (g)	Nestling Weight (g)
Number of Reference Samples (n)	2	6
Number of Target Samples (n)	9	11
Reference Mean	5.93	46.9
Target Mean	5.49	48.2
Reference Standard Error	0.305	4.61
Target Standard Error	0.194	3.32
Null Hypothesis (H_o)	$R_t = R_r$	$R_t = R_r$
p-Value	0.518	0.815
Power (alpha=0.05)	0.088	0.0557
Decision	Do not reject H_o	Do not reject H_o

Table 8
Measures of Effects: Survival to Fledging

	Nestling Period (days)	Range-Low Number of Nestlings Fledged per Successful Nest	Range-High Number of Nestlings Fledged per Successful Nest [a]	Fledging Success	Mayfield Nest Success
Number of Reference Samples (n)	9	11	11	11	338.5 exposure days
Number of Target Samples (n)	15	18	18	18	711 exposure days
Reference Mean	13.6	1.91	2.45	91%	10.7%
Target Mean	13.9	2.22	3.17	98%	26.4%
Reference Standard Error	0.176	0.163	0.247	0.0651	1.44%
Target Standard Error	0.206	0.129	0.0285	0.0183	0.8%
Null Hypothesis (H_o)	$R_t = R_r$	$R_t = R_r$	$R_t = R_r$	$R_t = R_r$	$R_t = R_r$
p-Value	0.221	0.145	0.00759	0.288	0.0650
Power (alpha=0.05)	0.226	0.305	0.794	0.242	NC
Decision	Do not reject H_o	Do not reject H_o	Reject H_o	Do not reject H_o	Do not reject H_o

NC - not calculated because measure not calculated by CONTRAST software
[a] number of nestlings predicted to have fledged if viable eggs and nestlings had not been collected
[b] value provided is standard error

Table 9
Spearman Correlation Analysis Based on Wet Weight Concentrations of PCBs

	PCB Concentration in Viable Eggs		PCB Concentration in Nestlings	
	Correlation Coefficient	Probability Level (p-value)	Correlation Coefficient	Probability Level (p-value)
PCB Concentration in Eggs	--	--	1.00	0
PCB Concentration in Nestlings	1.00	0	--	--
Hatching Success	0.191	0.651	0	1.00
Clutch Size	-0.300	0.370	0.288	0.263
Number of Young Died/Depredated	0.218	0.604	-0.153	0.557
Abandoned Nests	0	1.00	-0.153	0.557
Depredated Nests	0.299	0.372	0	1.00
Range-Low Number of Young Hatched	0.247	0.555	-0.283	0.270
Range-High Number of Young Hatched	0.193	0.619	0.0423	0.891
Range-Low Number of Young Fledged	0.316	0.489	-0.246	0.358
Range-High Number of Young Fledged	-0.425	0.255	0.373	0.209
Egg Date	0.800	0.200	-0.459	0.300
Incubation Period	-0.686	0.324	0.179	0.701
Fledging Period	0.309	0.552	0.245	0.379
Number of Nonviable Eggs	-0.535	0.138	0.271	0.292

Table 10
Spearman Correlation Analysis Based on Lipid-Normalized Concentrations of PCBs

	Lipid Normalized PCB Concentration in Eggs		Lipid Normalized PCB Concentration in Nestlings	
	Correlation Coefficient	Probability Level (p-value)	Correlation Coefficient	Probability Level (p-value)
PCB Concentration in Eggs	--	--	1.00	0
PCB Concentration in Nestlings	1.00	0	--	--
Hatching Success	0.027	0.949	0	1.00
Clutch Size	-0.200	0.555	0.199	0.443
Number of Young Died/Depredated	0.187	0.657	-0.153	0.557
Abandoned Nests	0	1.00	-0.153	0.557
Depredated Nests	0.179	0.598	0	1.00
Range-Low Number of Young Hatched	0.0825	0.846	-0.220	0.395
Range-High Number of Young Hatched	0.0826	0.833	0.127	0.680
Range-Low Number of Young Fledged	0.316	0.489	-0.164	0.544
Range-High Number of Young Fledged	-0.390	0.300	0.450	0.123
Egg Date	1.00	0	-0.184	0.694
Incubation Period	-0.894	0.106	0.179	0.701
Fledging Period	0.154	0.770	0.249	0.371
Number of Nonviable Eggs	-0.277	0.471	0.114	0.664

Table 11
Bioequivalence Test Results

	Biologically Relevant Difference (δ)	Probability level (p-value)	Decision	Power
Range-Low Number of Nestlings Hatched	0.5	0.0271	Reject Ho	0.619
Range-High Number of Nestlings Hatched	0.5	0.00007	Reject Ho	0.996
Range-Low Number of Nestlings Fledged	0.5	0.000289	Reject Ho	0.984
Range-High Number of Nestlings Fledged	0.5	0.000019	Reject Ho	0.999
Hatching Success	0.192	0.000679	Reject Ho	0.960
Fledging Success	0.182	0.00144	Reject Ho	0.970

Table 12

Summary of 95% Upper Confidence Limits

	Units	Reference		Target	
		Mean	95% UCL	Mean	95% UCL
Viable Egg PCB Concentration	mg/kg	0.153	0.691	83.6	125
Nestling PCB Concentration	mg/kg	0.0372	12.2	11.9	271
Lipid Normalized Egg PCB Concentration	mg/kg	2.41	6840	2,127	3,279
Lipid Normalized Nestling PCB Concentration	mg/kg	1.82	516	523	14,826
Nests Abandoned	%	6.5%	12.7%	1.5%	3.9%
Nests Depredated	%	65%	77.1%	63%	73.1%
Nest Defense Index	unitless	90.5	148	93	150
Predator Accessibility Index	unitless	2.13	2.21	2.07	2.15
Clutch Size	unitless	3.31	3.50	3.56	3.88
Egg Weight	g	5.93	7.85	5.54	5.84
Number of Nonviable Eggs	unitless	0.421	0.780	0.455	0.701
Incubation Period	days	14.0	14.0	14.2	14.6
Range-Low Number of Nestlings Hatched	unitless	2.64	3.40	2.89	3.22
Range-High Number of Nestlings Hatched	unitless	2.73	3.15	3.22	3.47
Hatching Success	%	96%	103%	93%	104%
Nestling Weight	g	46.9	56.2	43.4	58.0
Number of Young Abandoned/Depredated	unitless	1.84	2.51	0.636	1.61
Fledge Period	days	13.6	13.9	13.9	14.3
Range-Low Number of Nestlings Fledged	unitless	1.91	2.35	2.22	2.50
Range-High Number of Nestlings Fledged	unitless	2.45	2.90	3.17	3.40
Fledging Success	%	91%	114%	98%	102%

Figure 1
Range-Low Number of Nestlings Fledged in Successful Nests
(Mean ± 1 Standard Error)

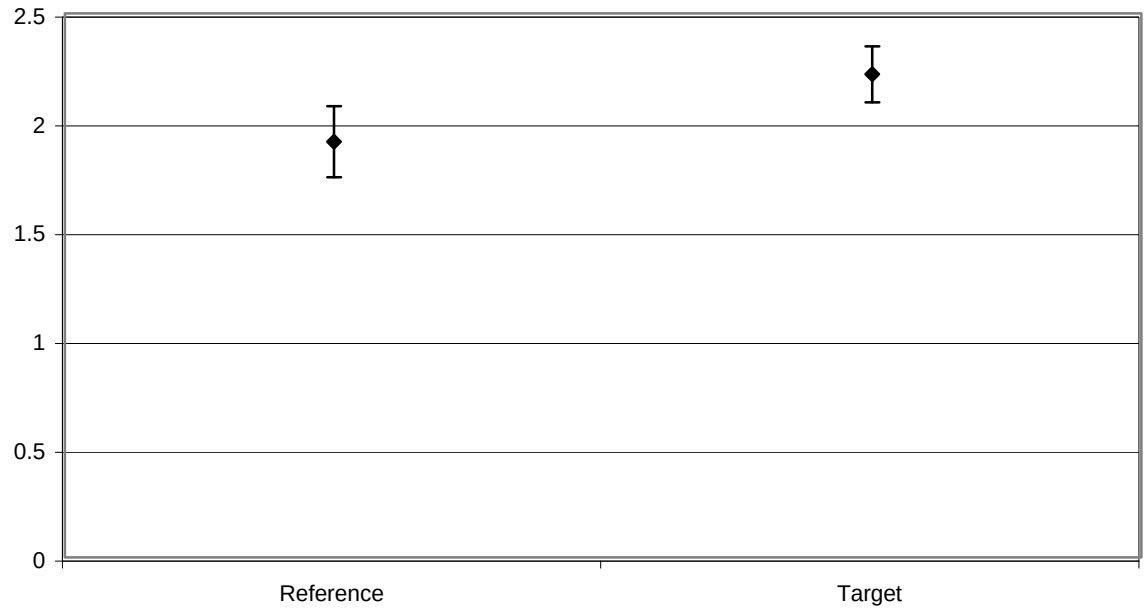

Figure 2
Range-High Number of Nestlings Fledged in Successful Nests
(Mean ± 1 Standard Error)

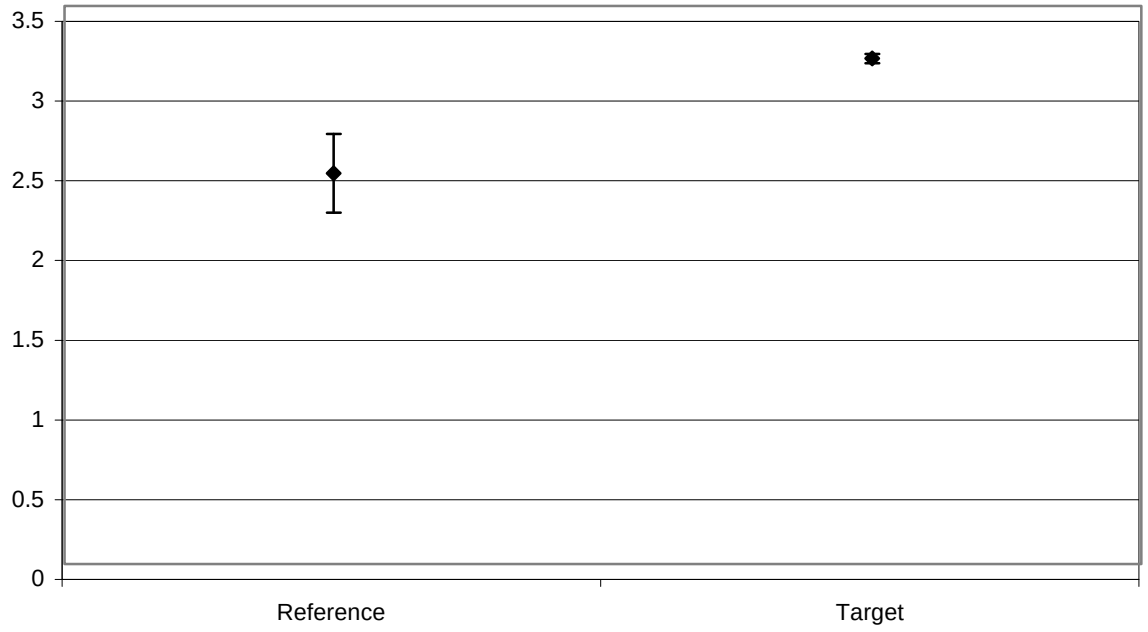

Figure 3
Fledging Success (Mean ± 1 Standard Error)

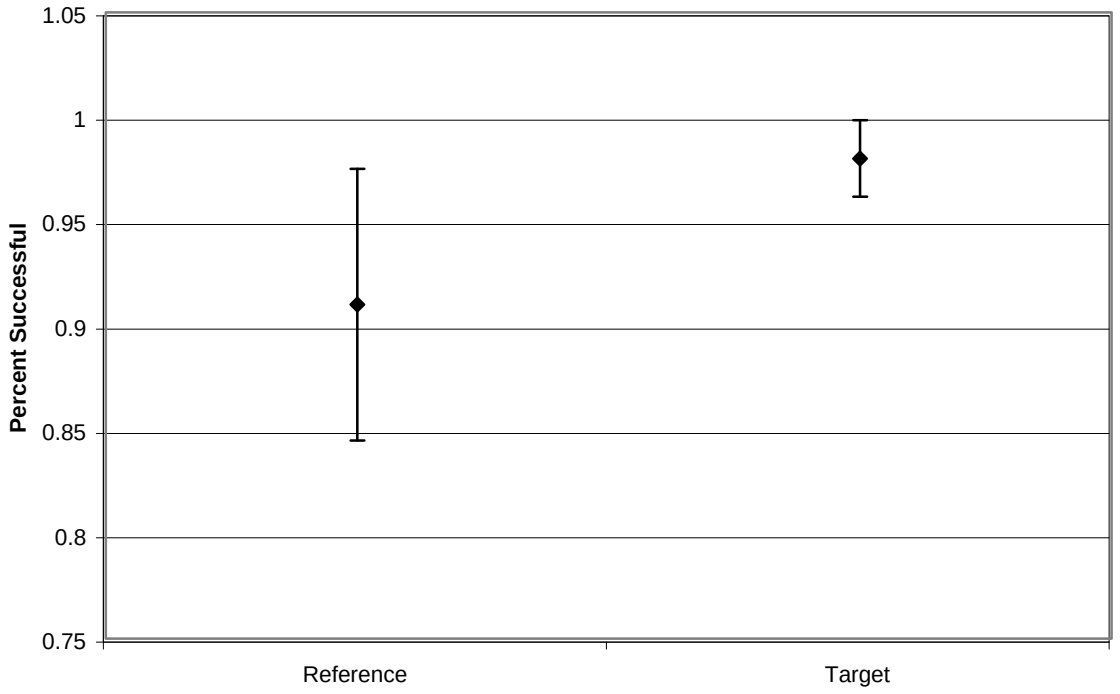

Figure 4
Mayfield Nest Success (Mean ± 1 Standard Error)